

AMENDMENTS TO THE CLAIMS:

Claims 32, 44, and 45 are canceled without prejudice or disclaimer. Claim 59 is amended. The following is the status of the claims of the above-captioned application, as amended.

Claim 1 (Original). A method for increasing the number of copies of an amplification unit integrated into a host cell chromosome, wherein the method comprises the steps of:

a) rendering a chromosomal gene of a host cell non-functional, wherein the host cell becomes susceptible to an inhibitory compound endogenously produced by the host cell when the host cell is cultivated in a medium comprising a precursor;

b) making a nucleic acid construct comprising an amplification unit, wherein the unit comprises:

i) an expression cassette comprising at least one copy of a gene of interest; and

ii) an expressible copy of the chromosomal gene of step a), wherein the unit integrates into the host cell chromosome;

c) introducing the nucleic acid construct of step b) into the host cell of step a), wherein at least one copy of the amplification unit integrates into the host cell chromosome;

d) cultivating the host cell of step c) in a medium comprising the precursor, wherein a chromosomally integrated copy of the amplification unit is duplicated or multiplied on the host cell chromosome;

e) selecting a host cell comprising two or more chromosomally integrated copies of the amplification unit; and optionally


f) performing one or more cycles of steps d) and e) using the host cell selected in step e) in each new cycle; wherein the number of chromosomally integrated copies of the amplification unit increases with each repeat.

Claim 2 (Original). A method for constructing a host cell comprising at least one copy of an amplification unit integrated into the host cell chromosome, wherein the method comprises the steps of:

a) rendering a chromosomal gene of a host cell non-functional, wherein the host cell becomes susceptible to an inhibitory compound endogenously produced by the host cell when the host cell is cultivated in a medium comprising a precursor;

b) making a nucleic acid construct comprising an amplification unit, wherein the unit comprises:

- i) an expression cassette comprising at least one copy of a gene of interest; and
- ii) an expressible copy of the chromosomal gene of step a), wherein the unit integrates into the host cell chromosome;
- c) introducing the nucleic acid construct of step b) into the host cell of step a) and cultivating the host cell in a medium comprising the precursor, wherein at least one copy of the amplification unit integrates into the host cell chromosome; and
- d) selecting a host cell comprising at least one chromosomally integrated copy of the amplification unit.

 Claim 3 (Canceled).

Claim 4 (Original). The method of claim 1, wherein the host cell is a *Bacillus* cell selected from the group consisting of *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*.

Claim 5 (Original). The method of claim 1, wherein the chromosomal gene of step a) encodes an enzyme selected from the group consisting of galactokinase (EC 2.7.1.6), UTP-dependent pyrophosphorylase (EC 2.7.7.10), UDP-glucose-dependent uridylyltransferase (EC 2.7.7.12), and UDP-galactose epimerase (EC 5.1.2.3).

Claim 6 (Canceled).

Claim 7 (Original). The method of claim 1, wherein the chromosomal gene of step a) is galE.

Claim 8 (Original). The method of claim 1, wherein the inhibitory compound is UDP-galactose.

Claim 9 (Original). The method of claim 1, wherein the precursor is free galactose.

Claim 10 (Original). The method of claim 1, wherein the precursor can be degraded to produce free galactose.

Claim 11 (Original). The method of claim 1, wherein the precursor is lactose, melibiose, raffinose, stachyose, verbascose or galactinol.

Claim 12 (Original). The method of claim 1, wherein the medium comprises an enzyme capable of degrading the precursor to produce free galactose.

Claim 13 (Original). The method of claim 1, wherein the host cell secretes an enzyme into the medium which is capable of degrading the precursor to produce free galactose.

Claim 14 (Original). The method of claim 12, wherein the enzyme is a galactosidase.

Claim 15 (Original). The method of claim 1, wherein the nucleic acid construct is a plasmid.

Claim 16 (Original). The method of claim 1, wherein the nucleic acid construct further comprises an antibiotic selection marker flanked by resolvase sites or res-sites.

Claim 17 (Original). The method of claim 1, wherein the amplification unit further comprises a nucleotide sequence with a homology to a chromosomal nucleotide sequence of the host cell sufficient to effect chromosomal integration in the host cell of the amplification unit by homologous recombination.


Claim 18 (Original). The method of claim 1, wherein the amplification unit further comprises a nucleotide sequence of at least 100 bp, preferably 200 bp, more preferably 300 bp, even more preferably 400 bp, and most preferably at least 500 bp with an identity of at least 70%, preferably 80%, more preferably 90%, even more preferably 95%, and most preferably at least 98% identity to a chromosomal nucleotide sequence of the host cell.

Claim 19 (Original). The method of claim 17, wherein the nucleotide sequence comprised in the amplification unit is a partial non-functional copy of a conditionally essential gene of the host cell, wherein the host cell prior to the first step of the invention has had the conditionally essential gene rendered non functional by a partial deletion, and wherein a recombination event between the partial copy of the gene comprised in the amplification unit and the partial chromosomal gene restores a functional chromosomal gene.

Claim 20 (Original). The method of claim 19, wherein the conditionally essential gene encodes a D-alanine racemase.

Claim 21 (Canceled).

Claim 22 (Original). The method of claim 1, wherein the amplification unit further comprises an antibiotic marker, preferably flanked by resolvase sites or res-sites.

 Claim 23 (Original). The method of claim 22, wherein a host cell comprising a first chromosomally integrated amplification unit is selected and the antibiotic marker excised from the host cell chromosome by a resolvase prior to the next step in the method.

Claims 24 – 58 (Canceled).

Claim 59 (Currently amended). A process for producing a polypeptide of interest, wherein the process comprises a step of cultivating a host cell comprising two or more chromosomally integrated copies of the amplification unit constructed by a method as defined in claim 1 of claim 45.

Claim 60 (Cancelled).

Claim 61 (Original). The process of claim 59 wherein the polypeptide is a hormone, a pro-hormone, a pre-pro-hormone, a small peptide, a receptor, or a neuropeptide.